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DATA EVALUATION REPORT

STUDY TYPE: 12 month dog (§83-1) TOX. CHEM. NO.: 557C

ACCESSION NUMBER: 265760, 265761 MRID NO.: N/A

TEST MATERIAL: 4-chloro-2-methyl-phenoxy acetic acid; technical

SYNONYMS: MCPA

STUDY NUMBER(S): 33D0046/8341

SPONSOR: Industry Task Force on MCPA research data

TESTING FACILITY: BASF Aktiengesellschaft, Abteiling Toxicology, Department Toxicology, D6700 Ludwigshaften

TITLE OF REPORT: Report on the study of the toxicity of MCPA in beagle dogs after 12-month administration in the diet (Project No. 33D0046/8341)

AUTHOR(S): Dr. med. vet. Hellwig (study director)

REPORT ISSUED: October 13, 1986

CONCLUSIONS:

In conclusion, the oral administration of technical MCPA to male and female beagles at doses of 0, 6, 30 and 150 ppm for a period of 52 week resulted in kidney and liver toxicity at the mid and/or high dose levels with alterations in related compounds or enzymes (kidneys: urea, potassium, creatinine; liver: bilirubin, GPT, GOT, triglycerides and cholesterol) associated with concommitant organ weight changes (liver) and histopathology changes (kidney: proximal tubular epithelium; liver: change in nature of gall fluid). Therefore, based upon kidney and liver toxicity of a moderate nature at the 30 and 150 ppm dose levels, the sytemic toxicity NOEL is set at the 6 ppm (LDT).

This study is designated as Core Minimum data.

A. MATERIALS: (a photocopy of the material and methods is attached)

1. Test compound: 4-chloro-2-methyl-phenoxy acetic acid, Description: solid, yellowish brown, Batch # 83/46, Purity 94.8%

2. Test animals: Species: dog, Strain: beagle, Age: 4-9 mos, Weight: males = 6.9 kg(mean); females = 7.3 kg(mean), Source: BASF's breeding facilities.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned 6/sex/dose to the following test groups:

Test Group	Dose in diet: (ppm)	mg/kg* male	mg/kg* female	Main Study 12 months male	Main Study 12 months female
1 Cont.	0	0.00	0.00	6	6
2 Low (LDT)	6	0.20	0.21	6	6
3 Mid (MDT)	30	1.02	1.02	6	6
4 High(HDT)	150	5.31	5.12	6	6

* average of mean values for four sampling periods (13 week intervals)

2. Diet preparation

Diet was prepared every 2 weeks and stored at room temperature. Three samples of treated food (each dose) were analyzed for stability, concentration, and homogeneity before and then at approximately 3-month intervals. Additional investigations were conducted after about 4, 6 and 12 weeks into the study.

Results -

The test substance was stable in the feed. Mean analytical values at 0, 1, 10 and 32 days of storage at room temperature in a sample presented of a nominal test concentration of 30 ppm were 31.9 (106%), 31.6(105%), 31.6(105%) and 32.2(107%), respectively.

The range of mean values(%) of test concentrations from samples from various times are presented below. They are acceptable(15-20% of nominal).

Date sample taken	test group (ppm)	% of nominal
Jan. 24, 1984	6, 30, 150	99, 90, 105
Mar. 9, 1984	6, 30, 150	92, 101, 97
Mar. 8, 1984	6, 30, 150	105, 86, 91
Sept. 7, 1984	6, 30, 150	98, 88, 95

3. Animals received food (350gm/day mixed with 350 ml of water into a paste). Water was available ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

1) Clinical examiniations and pathology: means and standard deviations were calculated for body weights, body weight changes, fasted body weights, absolute and relative organ weights. T test (Williams) for simutaneous comparisons of several dose groups and the control was used with significance at $p<0.05$, 0.01.

2) Blood and plasma examinations: mean and standard errors were tabulated and individual dose groups were compared against the control using the t test. Statistical significance was $p<0.05$ and $p<.01$.

3) Urinalyses were assessed with the Chi^2 test in appropriate two-by-two contingency tables. Statistical significance was $p<0.05$ and $p<.01$.

5. Quality assurance inspections were performed during the study and the final report was examined also (total 15 inspections). A signed copy of the QAU statement was included dated Oct. 13, 1986.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice a day for signs of toxicity and mortality. On appearance of any clinical signs of toxicity they were checked several times daily.

Toxicity/Mortality (survival)

Individual clinical signs data were not submitted. No deaths were reported during the study (pg. 46). No compound-related clinical signs of toxicity were reported. Diarrhea or thin feces (frequently noted in the first two months on test) and vomiting (high frequency noted during 8-11 months on test) were reported and stated to be without dose-dependence.

2. Body weight

All animals were weighed once a week-(Wednesday) towards 9:30 a.m. (See summary table below for body weight data).

There was no compound-related effect of MCPA upon female body weights or body weight gains at any dose level during test material administration. Statistically significant depressions in body weights and body weight gains were noted in the 150 ppm dose level as compared to controls. Mean body weights were depressed around day 56 (9.03 kg/control vs 8.75 kg/HDT) with statistically significant changes reported by day 140 (10.53 kg/control vs 9.78 kg/HDT). This depression was noted thoughout the entire test period. Mean body weight gains were statistically signficant by day 42 (day 1-day 42)(1.88 kg/control vs 1.45/HDT) with mean body weight gains decreased in the HDT as compared to controls throughout the subsequent test substance exposure period.

3. Food consumption and compound intake

Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

Males consumed 100% of the diet during the entire period of the study. Females generally consumed all of the test diet but from the 8th week onward individual females exhibited decreased appetite to varying degrees and frequencies. The decreased appetite was generally found in the MCPA groups (30 ppm: No. 37-40; 150 ppm: 43, 45, 47). The mean food consumption for the females was 100%*(0 ppm), 99%(6 ppm), 96.4% (30 ppm) and 97.5%(150 ppm). This is probably a compound-related effect since the majority of these animals are in the MCPA dose group at the higher dose levels.

* one animal occasionally did not consume all of its feed

Selected Mean body weights (kg) (from Tables 5-22)

(ppm/ day)	mean b. wt. (kg/dog) at days: 0	28	56	84	112	140	161	210	252	287	364
males											
0	6.82	8.05	9.03	9.75	10.22	10.53	10.53	10.48	10.67	10.90	11.30
6	6.85	8.02	8.82	9.53	9.93	10.22	10.32	10.50	10.70	10.97	11.27
30	7.03	8.18	9.05	9.70	9.97	10.23	10.33	10.32	10.48	10.65	10.95
150	7.05	8.07	8.75	9.35	9.57	9.78*	9.77*	9.78	9.97	10.13	10.52
females											
0	7.20	8.02	8.65	8.90	9.28	9.52	9.73	9.87	10.12	10.23	10.77
6	7.43	8.17	8.68	9.05	9.33	9.68	9.72	9.95	10.33	10.30	10.78
30	7.40	8.25	8.82	9.07	9.57	9.93	9.97	10.05	10.37	10.68	10.95
150	7.30	8.10	8.72	9.03	9.35	9.62	9.73	9.90	10.28	10.42	10.87

* statistically significantly different (p<0.05)

Selected Mean body weight changes (kg) (from Tables 23-40)

(ppm/ day)	mean b.wt change (kg/dog) for days: 1-42	1-91	1-126	1-161	1-203	1-280	1-322	1-364
males								
0	1.88	2.95	3.67	3.73	3.65	3.98	4.30	4.48
6	1.67	2.70	3.30	3.47	3.55	4.10	4.32	4.42
30	1.75	2.62	3.17	3.30	3.17	3.57	3.87	3.92
150	1.45*	2.28*	2.70*	2.72*	2.63	3.02	3.23	3.47
females								
0	1.17	1.72	2.13	2.48	2.62	2.90	3.22	3.52
6	1.07	1.70	2.10	2.28	2.52	2.83	3.07	3.35
30	1.25	1.70	2.32	2.57	2.65	3.17	3.40	3.55
150	1.27	1.80	2.25	2.43	2.67	3.02	3.43	3.57

* statistically significantly different (p<0.05)

4. Ophthalmological examinations

Performed at the beginning, after about 6 months and at the end of the exposure period. Their eyes were examined with a KOWA RC2 fundus camera. Individual opthalmic data not submitted. No ophthalmic findings noted.

5. Blood was collected 1 week before treatment and at 0, 13, 26 and 52 weeks for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood Clotting Measurements		
	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic and chronic studies

No compound-related changes were noted for any hematology parameter except partial thromboplastin time (PTT), which is presented below:

PTT(sec)	Sampling period:		
Dose (ppm)	13 weeks	26 weeks	52 weeks
Males:			
0	12.85	12.80	12.98
6	12.60	12.37	12.82
30	12.58	12.72	13.02
150	13.27	13.55	13.77
Females:			
0	13.47	13.05	12.57
6	13.25	13.35	13.53
30	13.43	13.40	12.82
150	14.65*	13.82	14.23**

*/** stat. signif. at $p<0.05$/$p<0.01$

There was a compound-related prolongation in partial thromboplastin time (seconds) in the 150 ppm dose level males and females which was statistically significant at 13 weeks and 52 weeks in the males [e.g., females: 12.98/control vs 13.77/HDT (52 weeks); males: 12.57/control vs 14.23/HDT (52 weeks)]. This may relate to the hepatotoxicity of MCPA since clotting time may be altered through the release of additional heparin from damaged hepatocytes.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorous*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
	Cholinesterase#	x	Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

A summary table of selected clinical chemistry values is presented below. Overall, females appeared to be somewhat more sensitive to MCPA administration.

Elevations in plasma potassium (mmol/L), urea (mmol/L) and creatinine (umol/L) in males and/or females observed are suggestive of a nephrotoxic effect of MCPA. In males, urea concentrations were elevated at both the mid and high dose levels while female urea values were elevated only at 150 ppm (e.g., males: p1- 4.954/control vs 6.181/30 ppm, 7.389/150 ppm; females: p1- 6.357/control vs 7.397/150 ppm).

Creatinine was elevated in both sexes (statistically significant in both sexes of the HDT) at the mid and high dose levels as compared to controls at all sampling periods (p1, p2, p3)(e.g., p1- males, 78.721/control vs 103.984/HDT; p1- females, 81.654/control vs 96.453).

Selected clinical chemistry findings (from Tables 5-10,35,36,39-42)

Dose(ppm)		Bilirub. (umol/L)	Urea (mmol/L)	Creatn. (umol/L)	K^+ (mmol/L)	GPT (U/L)	GOT (U/L)	TRIG (mmol/L)	CHOL (mmol/L)
Males									
0	p1	2.600	4.954	78.721	4.186	1.512	0.778	0.346	2.670
	p2	3.566	6.120	84.400	3.941	1.240	0.620	0.337	3.608
	p3	1.968	4.641	86.171	4.116	1.257	0.617	0.318	3.272
6	p1	3.136	5.278	79.735	4.262	1.118	0.748	0.303	2.879
	p2	3.224	5.521	79.488	4.122	1.317	0.677	0.350	2.799
	p3	1.898	4.358	78.617	3.987	1.500	0.690	0.314	3.561
30	p1	3.680*	6.181*	91.185	4.426	1.472	0.917	0.364	2.361
	p2	3.390	6.662	94.422	4.197	1.418	0.727	0.366	3.346
	p3	2.002	5.141	93.181	4.101	1.352	0.715	0.372	2.997
150	p1	3.277	7.389**	103.984**	4.328	2.768	1.038*	0.365	2.785
	p2	4.089	7.798*	112.140**	4.186	1.653	0.745	0.390	3.840
	p3	2.348	5.833	107.609*	4.180	1.875	0.755	0.376	3.214
Females									
0	p1	3.417	6.357	81.654	4.108	1.172	0.890	0.277	1.751
	p2	3.705	5.031	77.293	3.838	1.122	0.622	0.413	4.313
	p3	2.958	4.693	81.370	3.876	1.117	0.640	0.335	3.908
6	p1	3.703	5.955	80.308	4.180	1.027	0.797	0.319	2.171
	p2	3.625	4.667	78.766	4.049	1.040	0.587	0.367	4.167
	p3	3.018	4.894	86.513	4.195	1.073	0.640	0.345	3.229
30	p1	3.550	6.596	91.584	4.354	1.000	0.852	0.349*	2.358*
	p2	3.600	4.831	87.150*	4.035	0.963	0.608	0.381	5.165
	p3	2.892	5.487	92.038	4.158*	1.120	0.633	0.395	5.287
150	p1	3.878	7.397	96.453*	4.302	1.995	1.043	0.392**	2.742**
	p2	5.206¶	5.717*	94.029**	4.169*	1.548	0.615	0.511	5.595
	p3	3.733	5.147	102.954*	4.230**	1.823	0.687	0.395	4.650

p1, 2, 3 = sampling periods of 13, 26, 52 weeks; *,**statistically significant difference from control (p not stated); ¶ large S.E.
TRIG = triglycerides; CHOL = cholesterol

Potassium levels (mmol/L) were elevated only in females at both the mid and high dose levels (e.g., p3-3.876/control vs 4.158/30 ppm and 4.230/150 ppm; statis. significant at $p<0.05/0.01$).

Elevations in bilirubin (umol/L), GPT (U/L), GOT (U/L), triglycerides (mmol/L) and cholesterol (mmol/L) values in male and/or female beagles (primarily at the HDT) suggest compound-related liver damage. Bilirubin was elevated at all sampling periods in both sexes at the HDT (e.g, p2-males: 3.566/control vs 4.089; p2-females: 3.705/control vs 5.206/HDT).

Triglycerides and cholesterol were elevated only in females in the mid and high dose groups (statistically significant only

in pl sampling time) (e.g., TRIG, pl: 0.277/controls vs 0.349/30 ppm and 0.392/150 ppm; CHOL, pl: 1.751/control vs 2.358/30 ppm and 2.742/150 ppm).

GPT and GOT enzymes were elevated in the HDT of both male and female beagles at most sampling periods (e.g., males, pl, GPT: 1.512/control vs 2.768, GOT: 0.778/control vs 1.038/HDT; females, pl, GPT: 1.172/control vs 1.995, GOT: 0.890/control vs 1.043/HDT)

6. Urinalysis°

Urine was collected overnight from all animals about 1 week before the beginning of the study, and about 13, 26 and 52 weeks after the beginning of test compound administration. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen
		x	Nitrite

* Required for chronic studies
° Not required for subchronic studies

No unusual urine findings were observed in the control or test compound dose groups.

7. Organ weights

Selected organ weights (absolute, relative) are presented below.

Absolute and relative kidney weights were not significantly depressed in either sex at any dose level. There is a suggestion of a depression at the HDT in both absolute and relative liver weights of females but not males (i.e., females: 342 g/3.172, control vs 298 g/2.725, HDT, respectively). Female absolute brain weights were depressed (statistically significant) as were the relative brain weights at the HDT (80.21 g/0.749, control vs 72.08 g/0.670, HDT). The absolute and relative thyroid weights were increased in both sexes (statistically significant in males) at the HDT as compared to controls (e.g., males: 0.6725 g/0.0060, controls vs 0.9418 g/.0090, HDT). The increased thyroid weigts may relate to stimulation of thyroid follicular cell division via pituitary secretion (TSH?) (see discussion under non-neoplastic histopathology).

Mean absolute organ weights (g/dog)/relative organ weights (g/100g b.wt. (from Tables 59-62 of report)

ppm/ day-	sex	liver	kidneys	brain	thyroid
(main)					
0	M	359/	54/	82.36/	.6725/
		3.173	0.481	0.728	0.0060
6	M	361/	60/	79.69/	.8123/
		3.210	0.535	0.711	0.0073
30	M	318/	57/	83.52/	.8347/
		2.908	0.516	0.762	0.0076
150	M	330/	56/	76.79/	.9418**/
		3.131	0.537	0.732	0.0090**
0	F	342/	49/	80.21/	.7860/
		3.172	0.463	0.749	0.0074
6	F	314/	49/	75.40/	.8043/
		2.932	0.456	0.703	0.0075
30	F	288/	47/	75.02/	.8030/
		2.625	0.429	0.698	0.0075
150	F	298/	49/	72.08*/	.8712/
		2.725	0.450	0.670	0.0080

statistically significant (*/, $p<0.05$;$p<0.01$)

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	.Aorta*	xx	.Brain*†
x	.Salivary glands*	x	.Heart*	x	Periph. nerve*#
x	.Esophagus*	x	.Bone marrow*	x	Spinal cord (3 levels)*#
x	.Stomach*	x	.Lymph nodes*	x	.Pituitary*
x	.Duodenum*	x	.Spleen*	x	Eyes (optic n.)*#
x	.Jejunum*	x	.Thymus*		Glandular
x	.Ileum*		Urogenital	xx	.Adrenals*
x	.Cecum*	xx	.Kidneys*†		Lacrimal gland#
x	.Colon*	x	.Urinary bladder*		Mammary gland*#
x	.Rectum*	xx	.Testes*†	xx	.Parathyroids*††
xx	.Liver*†		Epididymides	xx	.Thyroids*††
x	Gall bladder*#	x	Prostate		Other
x	.Pancreas*		Seminal vesicle	x	Bone*#
	Respiratory	xx	Ovaries*†	x	Skeletal muscle*#
x	.Trachea*	x	.Uterus*	x	Skin*#
	.Lung*			x	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

a. Gross pathology

A summary of selected gross necropsy findings are presented below.

Gross necropsy did not reveal any apparent compound-related effects for the liver. Two/six females at the HDT were reported to have a red brown coloration of the gall fluid. High dose group males (4/6) and mid and high dose females (4/6, 6/6,respectively) had a dark brown coloration of the kidneys. An increase in the relative number of pituitary cysts were reported (1/6 in each dose group of males; 2/6, 1/6 in mid and high dose females) in the groups receiving MCPA as opposed to 1/6 only in control females. This suggests a possible effect upon the pituitary.

Selected gross pathology (from Tables 63,64)

		Incidence of changes							
		males				females			
Type of change	ppm:	0	6	30	150	0	6	30	150
LIVER									
-focal fatty infiltration		0	1	1	0	0	0	0	1
-dark spots (teleangectasia?)		0	1	0	0	0	0	0	0
-dark brown coloration		0	0	0	1	0	0	0	0
-focal adhesion to right kidney		0	0	0	0	0	0	0	1
GALL BLADDER									
-red brown coloration of gall fluid		0	0	0	0	0	0	0	2
KIDNEYS									
-dark brown coloration		0	0	0	4	0	0	4	6
PITUITARY									
-cyst		0	1	1	1	1	0	2	1

b. Microscopic pathology

1) Non-neoplastic

Selected histopathology findings are presented below.

There is a dose-related increase in kidney pigment deposition in the proximal tubular epithelium. In males, moderate pigmentation of the tubular epithelium appears similar among all dose groups but there is an apparent increase in the incidence of pronounced pigmentation at the HDT (3/6 vs 0/6 in controls). In females, there is an increase in the incidence of moderate tubular pigmentation at the mid and high dose levels as compared to controls (1/6, controls vs 4/6, 30 ppm and 4/6, 150 ppm).

Lung findings are variable with females possibly having a higher incidence of minimal perivascular and peribronchial infiltration in the mid and high dose groups (0/6, controls vs 2/6, mid and 1/6, high).

As noted in the discussion on gross findings, pituitary cyst incidences in either the mid or high MCPA dose groups appear to be elevated above control values (i.e., males: 2/6, controls vs 5/6, HDT; females: 3/6, controls vs 4/6, 30 ppm, 4/6, 150 ppm). Pituitary cysts were noted in the subchronic oral dog study (Accession numbers 256612-256614; Project # B77/1867; R 6478) at higher dose levels (around 300-1400 ppm). In connection with this observation, it should be noted that one HDT male beagle had focal hyperplasia of the thyroid follicles. There may be some physiological relationship, i.e., through the hypothalmic-pituitary-thyroid feedback loop.

D. DISCUSSION:

MCPA (4-chloro-2-methyl-phenoxyacetic acid; technical) was administered in the diet of male and female beagles (6/sex/dose) at 0, 6, 30 and 150 ppm for 52 weeks.

No mortality or evidence of morbidity from compound administration in either sex was reported. Male but not female body weights were depressed at the HDT. Food consumption was minimally decreased in females at the mid and high dose levels (96.4% and 97.5% of controls, respectively).

MCPA is nephrotoxic and hepatotoxic based on effects upon serum enzyme levels, organ weight changes and histopathology findings. The thyroids, in conjunction with the pituitary gland, appear to be possible target organs also.

Urea, potassium and creatinine concentrations in the blood were elevated (often statistically significantly) in both sexes at both the mid and high dose MCPA levels (females only at both doses for K^+). These compounds are generally associated with kidney filtration malfunctions. Furthermore, there is increased pigmentation

Selected histopathology (from Tables 65,67)

Type of change ppm:	Incidence of changes males 0	6	30	150	females 0	6	30	150
KIDNEYS								
Pigment deposition in:								
-proximal tubular epithelium (total)	6	6	6	6	6	6	6	6
-proximal tub. epith.(minimal)	2	2	0	1	4	1	2	0
-proximal tub. epith.(slight)	2	2	3	0	1	4	0	2
-proximal tub. epith.(moderate)	2	2	3	2	1	1	4	4
-proximal tub. epith.(pronounced)	0	0	0	3	0	0	0	0
LUNGS								
-perivascular & peribronchial cell. infiltration, minimal	1	1	2	1	0	0	2	1
-septal thickening with fibrosis & activation of alveolar cells	0	3	2	2	1	2	3	1
PITUITARY								
-cyst(s)	2	1	2	5	3	1	4	4
Thyroids:								
-focal hyperplasia of follicles	0	0	0	1	0	0	0	0

(DISCUSSION continued)

of the proximal tubular epithelium in HDT males (pronounced) and in mid and high dose females (moderate).

Elevations in plasma bilirubin, GPT, GOT, triglycerides and cholesterol in both sexes at the mid and/or high dose level (primarily at the HDT in males; statistically significant only at the 13 week analyses for triglycerides and cholesterol) were also found. These clinical chemistry findings are associated with a small depression in female liver weights (HDT) and an apparent increase in red brown coloration of the gall fluid in HDT females-which may relate to possible alterations in the hepatic function.

In conclusion, the oral administration of technical MCPA to male and female beagles at doses of 0, 6, 30 and 150 ppm for a period of 52 week resulted in kidney and liver toxicity at the mid and/or high dose levels with alterations in related compounds or enzymes (kidneys: urea, potassium, creatinine; liver: bilirubin, GPT, GOT, triglycerides and cholesterol) associated with concommitant organ weight changes (liver) and histopathology changes (kidney: proximal tubular epithelium; liver: change in nature of gall fluid). Therefore, based upon kidney and liver toxicity of a moderate nature at the 30 and 150 ppm dose levels, the sytemic toxicity NOEL is set at the 6 ppm (LDT).

Reviewed by: James N. Rowe, Ph.D. James N. Rowe 5/11/88
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Quang Q. Bui, Ph.D. Quang Bui 5/26/88
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DATA EVALUATION REPORT

STUDY TYPE: Subchronic rat (§82-1) TOX. CHEM. NO.: 557C

ACCESSION NUMBER: 259958 MRID NO.: N/A

TEST MATERIAL: 4-chloro-2-methyl-phenoxy acetic acid; technical

SYNONYMS: MCPA

STUDY NUMBER(S): 3150046/8302

SPONSOR: Industry Task Force on MCPA research data

TESTING FACILITY: BASF Aktiengesellschaft, Abteiling Toxicology, Department Toxicology, D6700 Ludwigshaften

TITLE OF REPORT: Report on the study of the toxicity of MCPA in rats after 3-months' administration in the diet

AUTHOR(S): Dr. med. vet. P. Kirsch (study director)

REPORT ISSUED: April 1, 1985

CONCLUSIONS:

MCPA technical was fed to male and female rats for 3 months at dose levels of 0, 50, 150 and 450 ppm. The kidneys were the major target organ based upon increased absolute and relative kidney weights in mid and high dose males, but not females. This was asssociated with a significant decrease in serum calcium in HDT males and a significant elevation in creatinine concentrations in HDT females. Both sexes had apparent crystalluria (oxalate, calcium phosphate, urate) in the high dose groups, another possible indicator of kidney dysfunction. These findings in the kidney were not confirmed from histopathological evaluation, suggesting that the effects are mild, perhaps more of a pharmacological than toxicological nature. Hepatotoxicity is also suggested in HDT males based upon prolongation in clotting times and decreased cholesterol concentrations. Based upon significant elevations in kidney weights (absolute, relative) at both the mid and high dose levels in male rats, the systemic toxicity NOEL is set at 50 ppm.

This study is designated as Core Minimum data.

A. MATERIALS: (a photocopy of the material and methods is attached)

1. Test compound: 4-chloro-2-methyl-phenoxy acetic acid, Description: solid, yellowish brown, Batch # 83/46, Purity 94.8%

2. Test animals: Species: rat, Strain: Wistar Chbb = THOM(SPF), Age: 42 days (at study initiation) Weight: males = 182.9 g(158-205g); females = 143.0 g(127-167g), Source: Dr. Karl Thomas GmBh, Biberach/Riss, FRG

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned 15/sex/dose to the following test groups:

Test Group	Dose in diet: (ppm)	mg/kg/day* male	mg/kg/day* female	Length: 3 months male	Length: 3 months female
1 Cont:	0	0.00	0.00	15	15
2 Low (LDT)	50	3.616	4.051	15	15
3 Mid (MDT)	150	10.855	12.134	15	15
4 High(HDT)	450	32.557	35.844	15	15

* average daily intake based upon total compound intake per kg b.wt. divided by 91 days

2. Diet preparation

Diet was prepared every week and stored at room temperature. Analyses of the test substance were carried out in several analytival laboratories before the beginning of the study. The content of the active ingredient and impurities was determined. The homogeneity and stability of the test substance diet formulations were performed prior to study initiation.

Results -

Homogeneity in feed of MCPA was tested (5 samples/dose; March 17 or 25, 1983). Mean % nominal concentrations for 50, 150 and 450 ppm dose levels were 116%, 105% and 90%, respectively. Concentration ranges were within ±20%.

The test substance (94.8% purity) was stable in the feed. Mean analytical values (%) at various days of storage at room temperature in a sample presented below (note: three samples/dose/time period were analyzed):

Nominal Dose	Time period (days): 0	14	17	18	28
50 ppm	114%	----	----	104%	104%
150 ppm	103%	105%	----	99%	101%
450 ppm	92%	----	97%	----	104%

3. Food administration - Animals were served Kliba 343-A-Rat/mouse maintenance diet of Klingental-muhle AG, CH-4303 Kaiseraugst ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

1) Clinical examinations and pathology: means and standard deviations were calculated for food intake, body weights and body weight changes. Standard deviations were calculated for exsanguinated body weights, and absolute and relative organ weights. T test (Williams) for simultaneous comparisons of several dose groups and the control was used with statistical significance at $p<0.05$ and 0.01.

2) After statistical correction (Nalimov criterion, 1963) blood and plasma examination mean and standard errors were tabulated and individual dose groups were compared against the control using the t test. Statistical significance was $p<0.05$ and $p<.01$.

3) Urinalyses were assessed with the Chi^2 test in appropriate two-by-two contingency tables. Statistical significance was $p<0.05$ and $p<.01$.

5. Quality assurance inspections were performed during the study and the final report was examined also (total 9 inspections). A signed copy of the QAU statement was included dated April 4, 1985.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected each day for signs of toxicity. Each time the animals were weighed they were also inspected and palpated. A check for moribund or dead animals was performed twice each day (Mondays to Fridays) or once each day on weekends and holidays.

Toxicity/Mortality (survival)

Individual clinical data were not presented. No clinically signs of compound-related toxicity were reported for any animal of either sex during the administration period.

No animals of either sex died during the study period.

2. Body weight

All animals were weighed once a week (Wednesday).

Initial and final body weights are presented below. No compound-related effects upon body weight or body weight changes were noted for either sex.

dose group(ppm)	day 0	day 49 (gms b. wt.)	day 91	B. wt gains (gm) (day 1-91)
males				
0	180.27	385.40	457.27	277.00
50	182.93	395.53	469.00	286.07
150	184.87	398.73	470.53	285.67
450	183.47	384.60	451.20	267.73
females				
0	142.73	241.93	275.13	132.40
50	140.60	237.60	266.93	126.33
150	141.20	240.13	266.47	125.27
450	147.33	245.60	272.93	125.60

3. Food consumption and compound intake

Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

No significant effect of MCPA ingestion upon food consumption was noted in either sex at any dose level.

4. Ophthalmological examinations

Performed at the beginning and at the end of the exposure period with focusable hand slit lamp for any changes to the refracting media. Any changes were photographed with a KOWA RC2 fundus camera. Individual opthalmic data were not submitted.

No impairment of the refracting media or ocular fundus in control or MCPA-treated animals was reported.

5. Blood was collected at in randomized sequence at 43 and 85 days for hematology and clinical analysis from ten animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood Clotting Measurements (Thromboplastin time)		
x	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Mean leukocyte counts were the only hematologic values which appeared altered during the study (see below):

Dose(ppm) Males		Giga/L	Dose(ppm) Females		Giga/L
0	p1	6.438	0	p1	5.053
	p2	5.926		p2	4.134
50	p1	6.850	50	p1	4.734
	p2	6.755*		p2	4.139
150	p1	7.070	150	p1	4.544
	p2	6.776**		p2	4.055
450	p1	7.678*	450	p1	4.825
	p2	6.703**		p2	4.402

p1, p2 = sampling period, days 43, 85; from Tables 047, 048
*, ** = statistically significant ($p<0.05$, 0.01, resp.)

Mean male leucocytic counts were significantly elevated over control values ($p<0.05$ or 0.01) in all dose groups but primarily in the HDT group. No significant change in leukocyte counts were observed in the females rats.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

A summary table of selected clinical chemistry values is presented below.

Findings are variable for the males and females and are generally observed only in the high dose groups. Clotting time (HQT) was prolonged in high dose males at both sampling periods (p1: 36.6 sec, control vs 40.7 sec, HDT; p2: 37.9 sec/control vs 39.8 sec, HDT). Calcium and cholesterol concentrations were significantly decreased in HDT males (e.g., Ca^{2+}: p1, 2.791 U/L, control vs 2.660 U/L, HDT; Chol., p2: 1.642 mmol/L, control vs 1.467 mmol/L, HDT). Creatinine, GPT and GOT were not significantly affected.

HDT females had a significant elevation in creatinine at the second sampling period as compared to controls (53.745 umol/L, control vs 59.444 umol/L, HDT). Calcium, cholesterol and HQT appeared unaffected. Although not statistically significant, GPT and GOT (p2 only) appeared somewhat elevated (e.g., p1, GPT: 0.782 U/1, control vs 0.853 U/L, HDT; p1, GOT: 1.601 U/L, control vs 2.104 U/L, HDT).

Selected clinical chemistry findings (from Tables 03,04,017,018, 023-026,029-032)

Dose(ppm)		HQT (seconds)	Creatn. (umol/L)	Ca^{+} (mmol/L)	GPT (U/L)	GOT (U/L)	CHOL (mmol/L)
Males							
0	p1	36.600	48.321	2.791	0.811	1.807	1.523
	p2	37.944	50.631	2.658	0.778	1.520	1.642
50	p1	36.810	51.831	2.855	0.819	2.083	1.741
	p2	38.590	50.006	2.630	0.804	1.445	1.775
150	p1	35.844	50.183	2.797	0.819	2.047	1.559
	p2	37.856	49.909	2.653	0.856	1.524	1.675
450	p1	40.678*	50.102	2.660**	0.861	1.681	1.109
	p2	39.833	51.076	2.580*	0.829	1.525	1.467*
Females							
0	p1	34.767	53.745	2.630	0.782	1.601	1.296
	p2	31.590	52.207	2.542	0.736	1.548	1.600
50	p1	34.800	54.787	2.706	0.744	1.789	1.635
	p2	34.900	52.131	2.551	0.782	1.727	1.558
150	p1	34.967	53.999	2.642	0.770	1.748	1.447
	p2	33.370	53.794	2.519	0.724	1.480	1.469*
450	p1	35.750	52.630	2.646	0.853	2.104	1.179
	p2	33.533	59.444**	2.540	0.808	1.597	1.663

p1, 2 = sampling periods of 43, 85 days; *,**statistically significant difference from control ($p < 0.05$, 0.01, respectively)

6. Urinalysis°

Urine was collected overnight from ten animals/test group/sex at day 37 and 79 of the study after the beginning of test compound administration. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen
		x	Nitrite

* Required for chronic studies
° Not required for subchronic studies

Selected urinalysis values are presented below:

Urinalyses parameters[§]

Males	0 ppm	50 ppm	150 ppm	450 ppm
(C1)				
leukocytes (>+)	0	1	0	3
bacteria (+++)	1	0	3	2
(C2)				
crystals 1 (+++)	0	1	0	3
bacteria (+++)	1	3	2	3
Females				
(C1)				
crystals 1 (+++)	0	2	1	4*
(C2)				
crystals 1 (+++)	0	0	0	2

C1, C2 = collection periods at day 37 and 79;
* statistically significant ($p<0.05$); § ten animals/dose group

The presence of leukocytes and bacteria in low and high dose males appears to be incidental since it is not dose-related. for the two collection periods (C1, C2). The observation of crystalluria in both HDT males (C2: 0/controls vs 3/HDT) and females (C1: 0/controls vs 4/HDT, statistically significant, $p<0.05$; C2: 0/controls vs 2/HDT) appears to be real and probably relates to an effect upon kidney filtration function.

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	.Aorta*	XX	.Brain*†
	.Salivary glands*	X	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*		Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary*
X	.Duodenum*	X	.Spleen*		Eyes (optic n.)*#
X	.Jejunum*	X	.Thymus*		Glandular
X	.Ileum*		Urogenital	XX	.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*		Mammary gland*#
X	.Rectum*	XX	.Testes*†		.Parathyroids*††
XX	.Liver*†		Epididymides	X	.Thyroids*††
	Gall bladder*#		Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*#
	Respiratory	XX	Ovaries*†	x	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung*			X	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

Eyes (optic n.) not examined as required by EPA test guidelines.

a. Organ weights

Selected organ weights (absolute, relative) are presented below.

There was a dose-related increase (statistically significant) in male absolute (g/rat) and relative kidney weights (g/100 g b. wt.) in the mid and high dose groups as compared to the controls (2.623/0.633, control vs 2.845/0.668, MDT and 2.956/0.724, HDT). This was not accompanied by any apparent histopathological findings. Absolute brain weights in males were also increased in the mid and high dose groups as compared to controls but this may not be a compound-related toxicity since it is not dose-related. The relative brain weights were similar to control values although slightly higher. No significant change in female kidney or brain absolute or relative weights were observed.

Mean absolute organ weights (g/rat)/relative organ weights (g/100g b.wt. (from Tables 045-048 of report)

ppm/ day-	sex	kidneys	brain
(main)			
0	M	2.623/ 0.633	1.861/ 0.450
50	M	2.743/ 0.649	1.941/ 0.462
150	M	2.845* 0.668	1.977/* 0.468
450	M	2.956/** 0.724**	1.931/* 0.473
0	F	1.887/ 0.772	1.824/ 0.747
50	F	1.816/ 0.764	1.785/ 0.752
150	F	1.889/ 0.791	1.832/ 0.769
450	F	1.949/ 0.798	1.835/ 0.756

*/**statistically significant ($p<0.05$;$p<0.01$, respectively)

b. Gross necropsy

No gross findings of an apparent compound-related nature were reported.

c. Histopathology

Selected histopathology findings are presented below.

In general, there is no evidence of compound-related toxicity in the organs and tissues examined. In the liver there are a number of males with reports of diffuse fatty infiltration with moderate peripheral infiltration but these are not dose-related effects. Adrenal cortical nodular hyperplasia is also reported in both males and females but not in any pattern suggestive of MCPA-induced toxicity. One HDT male had diffuse tubular atrophy of the testes in conjunction with diffuse hyperplasia of the Leydig cells. Also one HDT female had a small ovarian cyst. The biological occurrence of these observations does not seem associated with MCPA administration although atropy of the testes and ovaries in mice at a higher dose (2700 ppm) in a four week dose-ranging study has been reported (Accession No. 259957).

Selected histopathology (from Tables 050-052)

Type of change ppm:	Incidence of changes[a] males 0	50	150	450	females 0	50	150	450
LIVER								
-diffuse fatty infiltration, with peripheral intensification (slight)	0	0	1	1	1	0	0	1
-diffuse fatty infiltration, with peripheral intensification (moderate)	1	2	0	3	0	0	0	0
ADRENALS								
-cortical nodular hyperplasia	1	3	0	0	1	0	1	0
URINARY BLADDER								
-diffuse epithelial hyperplasia, minimal	0	-	-	1	0	-	-	0
TESTIS								
-diffuse tubular atrophy, very strong	0	-	-	1				
-diffuse hyperplasia, interstitial cells of Leydig, strong	0	-	-	1				
OVARY								
-small cyst					0	-	-	1

[a] 15 animals/sex examined

DISCUSSION:

MCPA technical was fed to male and female rats for 3 months at dose levels of 0, 50, 150 and 450 ppm. MCPA administration produced no clinical signs of toxicity, mortality, depression in body weight gains, decrease in food consumption or opthalmological findings. The kidneys appear to be the major target organ based upon increased absolute and relative kidney weights in mid and high dose males, but not females. This was asssociated with a significant decrease in serum calcium in HDT males and a significant elevation in creatinine concentrations in HDT females. Both sexes had apparent crystalluria (oxalate, calcium phosphate, urate) in the high dose groups, another possible indicator of kidney dysfunction. These findings in the kidney were not confirmed from histopathological evaluation, suggesting that the effects are mild, perhaps more of a pharmacological than toxicological nature. Hepatotoxicity is also suggested in HDT males based upon prolongation in clotting times and decreased cholesterol concentrations.

Based upon significant elevations in kidney weights (absolute, relative) at both the mid and high dose levels in male rats, the systemic toxicity NOEL is set at 50 ppm.

13544

R058656

Chemical:	**MCPA (and salts and esters)**
PC Code:	**030501**
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/26/88 12:00:00 AM**
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